Appendix B-1

Dioxin Assay

1.0 PURPOSE

To collect blood samples for dioxin testing in accordance with Center for Disease Control standards.

2.0 SCOPE

Applies to all Air Force Health Study participants.

3.0 MATERIALS

3.1 Blood-pack unit without anticoagulant - 600 ml

3.2 Alcohol swabs

3.3 Sepps

3.4 Sterile gauze

3.5 Adhesive tape

3.6 Balance

3.7 Coban

3.8 Unit holders

4.0 PROCEDURE

4.1 On the second day of the study, blood is drawn from patient with a 15 gauge needle into a blood pack unit without anti-coagulant.

4.1.1 Blood pack units have been previously tested by the CDC for Dioxin contamination.

4.2 Patients who have immunology studies have 250 ml of blood drawn. Patients not having immunology studies have 350 ml of blood drawn.

4.3 Select site for venipuncture.

4.3.1 On patients who have not yet had their physical exam, the dominant arm is preferred.

4.4 Prepare site for venipuncture in accordance with CDC standards.

4.5 Perform venipuncture and securely tape needle and tubing to arm.

4.6 Blood is collected into unit bag.

4.6.1 Amount of blood collected is determined by weighing sample on a balance.

4.6.2 For 280 ml of blood, set balance at 320 gms
For 350 ml of blood, set balance at 390 gms

4.6.3 When amount needed is obtained clamp tubing with hemostat.

4.7 Remove needle from vein

4.8 Have patient apply pressure to site for several minutes.

4.9 Apply pressure bandage to site using gauge and Coban.

4.9.1 Instruct patient not to remove bandage for at least 30 - 45 minutes.

4.10 Clamp tubing twice with hand sealer and clips.

4.10.1 Cut tubing and discard

4.10.2 Dispose of needle in needle container

4.11 Label unit bag with pre-printed label.

4.11.1 Write time drawn and initials on label

4.11.2 Place label on plastic portion of unit pack

4.12 Place unit bag upright in vertical holder.

4.12.1 Vertical holders are numbered 1-37.

4.12.2 Units are placed in holders according to order of draw.

4.12.3 Units are to remain upright at room temperature and allowed to clot for at least 7 hours.

5.0 SHORT DRAWS

5.1 In the event of a short draw, unit pack is to be weighed and the amount of blood noted on the unit label. "Short draw" should also be written on label in large letters.

6.0 MUTIPLE VENIPUNCTURES

6.1 If unable to collect sample with one venipuncture, ask patient if he is willing to be drawn again. If patient is willing start procedure from beginning.

6.2 If patient is unwilling to be redrawn, notify the nurse coordinator and Air Force monitor.

6.2.1 Save labels and have test credited.

7.0 MAILING OF SAMPLES

7.1 Frozen samples are mailed twice weekly to Brooks AFB, TX via Airborne Overnight Service.

7.2 Mailing boxes are placed in styrofoam shipping tape.

7.2.1 10 - 15 lbs of dry ice is packed around mailing boxes.

7.3 CDC shipping list is placed on top of styrofoam lid and beneath cardboard box lid.

7.4 Cardboard box is sealed with strapping tape.

7.5 Address label, dry ice label and "this side up" label are placed on box.

7.6 Mailing requisition is filled out and taken with shippers to shipping department.

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POLICIES & PROCEDURES

Scripps Clinic

DEPARTMENT NAME: CLINICAL PATHOLOGY	P. P. NUMBER	ISSUE DATE:
TITLE: AIR FORCE HEALTH STUDY - DIOXIN BLOOD PROCESSING	REVISION DATE: 8/4/87	PAGE 1 OF 4

1.0 PURPOSE:

To process blood samples for dioxin testing using Center for Disease Control standards as a guideline.

2.0 SCOPE:

Applies to Clinical Pathology Medical Technicians involved in processing dioxin samples.

3.0 MATERIALS:

3.1 transfer pack units = 300 ml

3.2 plasma transfer set

3.3 plasma extractor

3.4 vertical holders

3.5 vertical holder boxes

3.6 teflon lined lids

3.7 teflon stoppers

3.8 aluminum sealing caps

3.9 aluminum cap sealer

3.10 centrifuge bags

3.11 hand sealer/stripper

3.12 shipping list

3.13 Wheaton bottles

3.13.1 5 ml, 10 ml, 120 ml

3.14 white storage bags

3.15 styrofoam mailing boxes

3.16 dry ice

4.0 PROCEDURE:

4.1 Shipping list

4.1.1 The shipping list is a modified version of the list provided by CDC.

4.1.2 Shipping list is prepared as follows; remove top left section of patients label from unit bag and place sequentially on shipping list.

4.1.3 Specify any deviations from collection, storage and shipment protocols, and date of occurrence.

4.2 Centrifuging of unit bags

4.2.1 Centrifuges are refrigerated at 4-10°C.

4.2.2 Unit bags are centrifuged in the order that they are drawn.

4.2.3 Unit bags are placed inside centrifuge bags and then in centrifuge cups.

4.2.3.1 Centrifuge cups are then balanced.

4.2.4 Centrifuge cups are placed in centrifuge and spun for 15 minutes at 4500 rpm.

4.2.5 Balance next group of 10 unit bags for centrifuging.

4.3 Transfer of serum from unit bags to transfer packs.

4.3.1 Label transfer packs with patients aliquot label.

4.3.2 Labeled transfer packs are placed in vertical holders in the sequence they are to be transferred.

4.3.3 Serum is transferred from spun unit bag to the transfer pack by plasma extractor.

4.3.3.1 Place unit bag on plasma extractor with side not containing manufacters label toward you.

4.3.3.2 Remove coupler cover of transfer pack unit.

4.3.3.3 Expose outlet port of blood pack unit.

4.3.3.4 Insert coupler into outlet port.

4.3.3.5 Release handle of plasma extractor and express desired amount of serum into transfer pack unit.

4.3.3.6 Apply clips and hand sealer to transfer tubing and severe tubing between seals.

4.3.4 Transfer packs containing serum and any unit bags that need to be respun are placed in unsequential vertical holders, that are placed in boxes 2A and/or 2B.

4.3.5 20 transfer packs are spun at a time.

4.3.5.1 Transfer packs spin for 15 min. at 4500 rpm.

4.4 Transfer of serum from transfer packs to Wheaton bottles.

4.4.1 Wheaton bottles are labeled with patient aliquot labels

4 oz Wheaton bottle	S1 Serum Dioxin
5 ml Wheaton bottle	S3 Lipid Profile
10 ml Wheaton bottle	S4 Serum Reserve
4 oz Wheaton bottle	S2 Serum Dioxin

4.4.1.1 Insert the sharp end into one of the outlet ports in top of the bag.

4.4.1.2 Close tubing with thumb roller on tubing.

4.4.1.3 Press bag with "Plasma Extractor".

4.4.1.4 Hold open end of tubing over pre-labeled Wheaton bottles.

4.4.1.5 Open tubing and put 5 ml in "S3" bottle, 10 ml in "S4" bottle and divide the rest into the 4 oz bottles.

4.4.1.6 Extract only the serum being careful that cells do not enter the bottle. Recap and tighten.

4.4.1.7 Log in the serum samples and store at -20°C or less until shipment.

4.4.1.7.1 Serum samples are stored in white cardboard boxes provided by C.D.C.